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chromatography", Journal of the Taiwan Institute of Chemical Engineers, 2014.


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Emmanuel Revellame, "Biodiesel from activated sludge through in situ transesterification", Journal of Chemical Technology & Biotechnology, 2009

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Seung Tae Hong. "Transesterification of palm oil using supercritical methanol with co-solvent HCFC-141b", Research on Chemical Intermediates, 02/2009


Submitted to University Der Es Salaam on 2015-06-17

Ong, Lu Ki, Phuong Lan Tran Nguyen, Felycia Edi Soetaredjo, Suryadi Ismadji, and Yi-Hsu Ju. "Direct reuse of Cu-laden wastewater for non-edible oil hydrolysis: basic mechanism of
Phuong Lan Tran-Nguyen a, Alchris Woo Go b,

Suryadi Ismadji c, Yi-Hsu Ju a,

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Highlights Produce biodiesel from activated sludge in subcritical methanol with acetic acid. Require much shorter time and much less methanol than acid catalyzed method. Less severe operation condition than that of supercritical methanol method.

In situ transesterification Subcritical solvents graphical abstract Transesterification Methanol Methanol AA Filtration + AA T (oC) = 250 Sludge Time (min) = 30 Methanol + AA+ FAME Methanol + Glycerol Biodiesel Separation abstract Most previous studies reported in literature
On biodiesel production from sludge were performed by acid catalyzed transesterification that required long reaction time (about 24 h) and high methanol loading. The objective of this study was to investigate the in situ transesterification of sludge in subcritical mixture of methanol and acetic acid. At 250 °C and a solvent (85% methanol and 15% acetic acid)

To sludge ratio of 5 (mL g⁻¹), a FAME yield of 30.11% can be achieved in 30 min, compared to the yield of 35% obtained by the acid-catalyzed (4% H₂SO₄) transesterification which required 24 h.

At 55 °C and a methanol to sludge ratio of 25 (mL g⁻¹). The method developed in this study avoided using mineral acid, significantly reduced reaction time and methanol loading to achieve comparable FAME yield. © 2015 Elsevier Ltd. All rights reserved.

1. Introduction Due to global warming caused by excessive emission of greenhouse gases, particularly carbon dioxide and the increasing energy demand, there is an urgent need to find alternative fuels to replace the traditional fossil-based fuels. Biodiesel is one of the candidates to solve the environmental pollution, reduce dependence on Abbreviations: AA, acetic acid; FAME, fatty acid methyl ester; FFA, free fatty acid; HTGC, high temperature gas chromatography; SCW, subcritical water. ñ

Corresponding author. Tel.: +886 2 27376612; fax: +886 2 27376644. E-mail address: yhju@mail.ntust.edu.tw (Y.-H. Ju). http://dx.doi.org/10.1016/j.biortech.2015.08.033 0960-8524/© 2015 Elsevier Ltd. All rights reserved. limited resources and increase the use of renewable resources. Biodiesel seems a very interesting alternative fuel for reasons such as it is renewable, less global warming than petroleum fuel, biodegradable and less toxic (Ahmad et al., 2012).

Biodiesel is mainly produced from transesterification reaction of vegetable oils or animal fats with alcohol, usually methanol in the presence of an acidic/basic catalyst, with glycerol as a co-product (Fan and Burton, 2009). Vegetable oil is the selected candidate of first generation biofuels in the past centuries (Mythili et al., 2014). Edible oils like soybean oil, sunflower oil, palm oil, rapeseed oil and
peanut oil are considered as the biodiesel raw materials. Atabani et al. (2012) claimed that more than 95% of

the world biodiesel is produced from rapeseed oil (84%), sunflower oil and palm oil (13% and 1%, respectively), and soybean oil and others (2%).

However, those vegetable oils are also used for human consumptions. It can result in an increase of food price, causing high biodiesel cost, fuel crisis, and environmental problems (Atabani et al., 2012; Mata et al., 2010). Moreover, depending on feedstock, biodiesel was sold at a price 1.5–3.0 times higher than that of petroleum diesel (Fan and Burton, 2009). In order not to compete with edible vegetable oils and reduce biodiesel price, low cost feedstock such as non-edible oils, used cooking oils, animal fats, soap-stocks, and greases should be considered as feedstock to produce biodiesel (Mata et al., 2010). Some recent studies have reported the potential of municipal sludge as biodiesel feedstock.

Mondala et al. (2009) obtained a maximum FAME yield of 2.5% from secondary sludge and estimated that using in situ transesterification the price of biodiesel from sludge ($3.23/gallon) could be lower than that of petroleum diesel ($4.80/gallon) and soybean biodiesel ($4.50/gallon).

Dufreche et al. (2007) estimated that the price of biodiesel could be $2.50/gallon at an overall sludge biodiesel yield of 10%, which is competitive with soybean biodiesel in the market. Revellame et al. (2011) reported a sludge biodiesel yield of 3.93% under the following conditions: reaction temperature 75 °C, ratio of methanol to sludge 30 mL g⁻¹, sulfuric acid concentration 10 wt.%, reaction time 24 h. In another study, they obtained a biodiesel yield of 4.79% in 24 h at 55 °C, a methanol to sludge ratio of 25 mL g⁻¹ using 4 vol.% sulfuric acid as the catalyst (Revellame et al., 2010).

Production of biodiesel from wet activated sludge
under subcritical condition at 175 °C was performed by Huynh et al. (2012). They reported a biodiesel yield of 45.58% in 8 h. To date, reports on biodiesel production from activated sludge are limited in literature. Most studies on biodiesel production from activated sludge employed conventional acid catalyzed transesterification which required long reaction time (24 h) and high ratio of methanol to dried sludge. There is no report on biodiesel production from activated sludge with the aim to reduce reaction time using subcritical mixture of methanol and AA. In this study, sub-critical methanol was used to shorten the reaction time. The effect of methanol loading was also investigated to minimize the amount of methanol required. The effect of adding AA on reducing reaction time and increasing FAME yield was studied.

2. Methods

2.1. Materials

Chemicals including methanol (99.9% purity), AA (99% purity), hexane (95% purity) were obtained from commercial sources and were of analytical reagent grade. Standards of FFAs, acylglycerides and FAMEs were purchased from Supelco (Bellfonte, PA). The activated sludge sample used in this study was collected from Hsin-Tung-Yang LTD Da-Yuan Factory which is a food processing plant. Water content of the wet sludge sample was 89.01% (Tran-Nguyen et al., 2013). The wet sludge was dried under sunlight. It was then ground and sieved. Powders that pass through standard mesh 24 (0.71 mm) but retained in mesh 60 (0.25 mm) were collected. The sludge powder was heated to 105 °C for 8 h to remove its residue moisture before use. The dried sludge was then stored at 20 °C before use. The moisture content of the dried sludge is 4.88%. Before biodiesel production, activated sludge was dried in an oven overnight and its moisture content was determined. Water content (MS) of a sludge sample was determined from the difference between weight of the sludge sample before and after drying at 105 °C, as shown in the following equation: MS = (WWS - WDS) / WS. 

2.2. In situ transesterification reaction

In situ transesterification reaction was performed based on the procedure of Huynh et al. (2012). Dried sludge and methanol either with AA or without AA, were loaded into a glass chamber (190 mL) and then placed in a high pressure reactor (290 mL). Experimental set up for in situ transesterification of activated sludge is depicted in Fig. 1. The reactor is equipped with an external electric heater. Stirring was provided by an external magnetic stirrer. Temperature in the reactor was controlled to within ±2 °C. After sample was put in the reaction chamber, the reactor was sealed and carefully insulated. The reactor was heated to the desired temperature in about 60 min at a heating rate of about 4 °C per min. The time the reactor temperature reached the desired temperature was set.
as time zero. After the reaction, the reactor was cooled to ambient temperature, the product was collected

and solid was separated by filtration. The solid was washed twice each with 50 mL methanol. The washed solution was pooled with the liquid phase from filtration. Solvent in the combined solution (methanol and AA) was removed by a vacuum evaporation

(BUCHI Labortechnik AG in Flawil, Switzerland) operated at 40 °C and 13.3 kPa to obtain the crude product. Hexane (100 mL) and salt solution (5% sodium chloride) (20 mL) were added into the separatory funnel to separate crude FAME from the aqueous phase and remove glycerol and residual acetic acid. The upper phase containing FAME was withdrawn and hexane was then removed by using a rotary evaporator. Crude FAME was weighed and its composition was analyzed by HTGC. At least two experiments were carried out for each reaction. The flow-chart of the in situ reaction is presented in Fig. 2. FAME yield is defined as the mass of FAME produced per mass of dried activated sludge used in the reaction and is calculated using

Eq. (2) Yield δ% = \( \frac{\text{Mass of FAME in the product (g)}}{\text{Mass of dry activated sludge (g)}} \times 100 \)

Determination of FAME yield by HTGC Before analyzing, 20 mg sludge FAME sample was dissolved in 1 mL ethyl acetate and filtered using magnesium sulfate to remove moisture. A 1 mL aliquot of ethyl acetate solution was injected in a Shimadzu GC-2010. External standard calibration curves were prepared by using 0.2–20 mg pure standards dissolved in ethyl acetate. A 37 components FAME mix standard was used to identify individual FAME in the product.

Chromatographic analysis was performed using a GC-2010 gas chromatograph (Shimadzu, Japan) equipped with a flame ionization detector. Separation was run on a ZB-5HT (5% phenyl) – methylpolysiloxane nonpolar column (15 m 0.32 mm i.d., 0.1 lm film thickness, Zebron, Phenomenex, Torrence, CA, USA). The carrier gas was nitrogen with a linear velocity of 30 cm s⁻¹ at 80 °C. The operating conditions were...
were set at 370 °C. The initial oven temperature began at 80 °C, increased to 365 °C at 15 °C min 1 and held for 8 min. The total analysis time was 29 min. The hydrogen flow, air flow and make up flow were set at 50.0 mL min⁻¹, 50.0 mL min⁻¹ and 30 mL min⁻¹, respectively while the linear velocity and purge flow were 8.0 cm s⁻¹ and 0.0 mL min⁻¹.

Data analyses were carried out by the software “GC Solution version 2.3”, Shimadzu.

726 V-8 3415 Fig. 1. Layout of equipments for in situ transesterification of activated sludge. [1] Nitrogen cylinder, [2] needle valve, [3] reactor, [4] heater, [5] magnetic stirrer, [6] pressure gauge, [7] thermo couple, [8] controller. Solid Hexane + salt solution (5% NaCl) Aqueous phase 3. Results and discussion 3.1. Characteristics of activated sludge Dry sludge Methanol + acetic acid Reaction Filtration Filtrate Vacuum evaporation Methanol Crude product Separation Crude FAME GC analysis Fig. 2. Flowchart of biodiesel production from dried sludge. Table 1 Characteristics of lipid in dry activated sludge before and after SCW treatment (Tran-Nguyen et al., 2013). Lipid mass fraction dry activated sludge (%) Table 1 shows the crude lipid content of AS before and after SCW treatment. Before treatment, the extractable lipid content Composition Before SCW pretreatment After SCW pretreatmenta was 17.99%, which was increased 2.54 times to 45.42% after SCW Unsaponifiable 0.50 5.75 treatment. In addition to increase of crude lipid, neutral lipid wax and gums 1.21 tent also increased 2.33 times after SCW treatment. This increase Neutral lipid 16.28 37.96 of extractable lipid after SCW treatment was the result of SCW a Pretreatment condition: 175 °C, 4 MPa, 15 min. hydrolysis of phospholipids which are microbial by-products resulting from microbial activity such as death and lysis in 3.2. FAME production from activated sludge wastewater treatment (Olkiewicz et al., 2015). SCW pretreatment can release lipids from other macromolecules which may not be Reaction temperature is the major parameter influencing trans- extractable. FFA content and fatty acids profiles of the activated esterification process. Therefore, in the preliminary investigation of sludge oil were reported by Tran-Nguyen et al. (2013). this

study, the effect of temperature on FAME yield was
investigated from 200 °C to 250 °C for 30 min with 30 mL methanol per gram dried sludge (data not shown). It was found that FAME yield was 7.32% at 200 °C and increased rapidly to 23.47% at 250 °C, an increase of about 3.2 times. It may be due to the increase of miscibility between methanol and oil thus an increase of reaction rate (Amit, 2012). Miscibility of methanol and oil are poor at room temperature. Reaction rate increased with increasing temperature owing to increase in solubility of methanol in the oil-rich phase. Higher reaction temperature results in shorter reaction time in mass transfer controlled reaction because esterification reaction is more preferred at higher temperature (Noureddini and Zhu, 1997). In addition, increasing temperature results in decreasing polarity of methanol and this leads to enhancing the solubility of fatty acids in methanol. Kusdiana and Saka (2004) also reported that in supercritical state, methanol acts as an acid catalyst in (trans)esterification reaction. A reaction temperature of 250 °C was chosen for all experiments in this study, which is a little higher than the critical temperature of methanol (239.6 °C); however, the reaction was still happened under subcritical condition because of the presence of lipids which have much higher critical temperatures. 3.2.1. Effect of reaction time on FAME yield Most of the studies were carried out at 50 to 75 °C for 24 h

6 (Dufreche et al., 2007; Mondala et al., 2009; Revellame et al., 2010, 2011).
Choi et al. (2014) produced

biodiesel from wet sludge with hexane as the co-solvent at 55 °C with

methanol to sludge ratio of 10 mL g⁻¹ and reported a FAME yield of 9.68% in 8 h.

4 Acid-catalyzed in situ transesterification of greasy sewage sludge

7 with methanol using hexane as the co-solvent was reported by Gerhard et al. (2015). A maximum methyl esters yield of 61% was achieved in 7 h at 55 °C and

methanol to sludge ratio of 20 mL g⁻¹. The effect of reaction time on FAME yield was investigated by carrying out reactions at 250
13°C with a methanol to dry sludge ratio of 30 mL g⁻¹ for up to 240 min. It was found that FAME yield increased with reaction time and reached a maximum of 28.98% at 120 min (Fig. 3), then decreased slightly to 25.70% as the reaction time was prolonged to 240 min. It was possibly the results of reverse transesterification and formation of fatty acids (Encinar et al., 2012; Eevera et al., 2009). Compared to conventional acid/base catalyzed reaction, in situ transesterification eliminates the oil extraction step, thus process productivity can be improved. In fact, a FAME yield of 28.98% was achieved in 120 min which is comparable to that of the study of Huynh et al. (2012) and conventional acid catalyzed transesterification but required 8 h and 24 h, respectively. However, by extending reaction time to 180 min and 240 min, FAME yield decreased slightly to 25.97% and 25.70%, respectively. This may be because the reaction has reached equilibrium.

3.2.2. Effect of methanol to sludge ratio on FAME yield

One of the most important parameters affecting the transesterification reaction is the mass ratio of methanol to biomass used in the reaction. Theoretically, transesterification requires three moles of methanol per mole of triglycerides.

The optimum molar ratio of methanol and Cynara oil to obtain high FAME yield by alkali catalyzed transesterification was between 4.05 and 5.67. Uncompleted reaction and difficult separation between methanol and glycerol occurred when molar ratio of methanol to triglycerides were less than 4.05 and higher than 5.67, respectively (Kusdiana and Saka, 2001; Encinar et al., 1999). In this study, the effect of methanol to dry sludge ratio on FAME yield was investigated by varying the ratio from 1:1 to 40:1 mL g⁻¹ at 250 °C for 120 min and the results are depicted in Fig. 4. It can be observed that FAME yield increased from 20.17% to 28.98% as methanol to dry sludge ratio was increased from 1:1 to 30:1 mL g⁻¹. Even at a methanol to dried sludge ratio of 1 mL g⁻¹, FAME yield could reach about 20%.
Biodiesel yield increased as more methanol was used. The best result was obtained at a methanol to dried sludge ratio of 30 mL g⁻¹. Since the transesterification is a reversible reaction, excess methanol is required to shift this equilibrium towards the formation of FAME (Encinar et al., 2012). Additionally, in the study of the kinetics of transesterification of rapeseed oil in supercritical methanol, it was reported that higher molar ratio of methanol to oil resulted in better FAME yield owing to the increase of contact area between oil and methanol (Kusdiana and Saka, 2001). However, further increase in methanol loading tended to have negative effects on FAME yield which decreased slightly to 25.56% as the ratio of methanol to dry sludge was increased to 40 mL g⁻¹. This is possibly the result of dilution effect as more amount of methanol was used. Another reason to avoid using too much methanol is that high methanol loading interferes with the separation between glycerol and FAME because of increase of glycerol in FAME as well as difficulty in washing step and 35 30 30 25 25 FAME yield (%) 20 15 FAME yield (%) 20 15 10 10 5 5 0 0 0 30 60 120 180 240 1.0 5.0 7.5 15.0 22.5 30.0 40.0 Reaction time (min) Ratio of methanol to dried sludge (mL.g⁻¹) Fig. 3. Effect of reaction time on FAME yield at 250 °C with a methanol to dry sludge ratio of 30 mL g⁻¹.

Fig. 4. Effect of methanol to dry sludge ratio on FAME yield. Reaction temperature = 250 °C, reaction time = 120 min. contamination of biodiesel product (Encinar et al., 2012). This trend is in agreement with the study of Huynh et al. (2012) which reported that a ratio of methanol to dry activated sludge higher than 30 mL g⁻¹ resulted in lower FAME yield. It may be because excess methanol favors extraction of more polar compounds, such as carbohydrate, proteins and pigments as well as the formation of mono-glyceride as explained in the Section 3.2.1. Excess methanol produces more glycerol which increases the driving force of reverse reaction. In addition, excess methanol increases the cost of methanol recovery. Following the study of Revellame et al. (2011), acid catalyzed transesterification of dry sludge was carried out at 55 °C by using 23a methanol to dried sludge ratio of 25 mL g⁻¹ and 4% H2SO4 (v/v). FAME yield was 11.38% at 2 h and reached a maximum value of 35.28% at 24 h. This maximum FAME yield is higher than the maximum FAME yield achievable (29%) in the subcritical methanol transesterification at 250 °C; however much shorter reaction time (2 h) was required in the latter case. Acid catalyst (H2SO4) is required in the former method which requires washing to remove acid from product. On the contrary, the latter process does not require the use of mineral acid.

A methanol to dry sludge ratio of 5 mL g⁻¹ was selected for the following study since increasing this ratio from 5 to 30 (an increase of 500%), FAME only increased from 25.83% to 28.98% (an increase of 12%). Furthermore, lowering the amount of methanol used is favorable to reduce the cost of biodiesel production. 3.3. Effect of AA on FAME yield Biodiesel productions from sludge using mineral acid, mostly H2SO4, as catalyst have been investigated by Dufreche et al. (2007), Mondala et al. (2009) and Revellame et al. (2010, 2011). However, there is no report on biodiesel production.
Production from activated sludge under subcritical methanol condition

with addition of AA, an organic acid.

At a solvent (methanol + AA) to dry sludge ratio of 5 mL g⁻¹, the effects of the amount of AA added on FAME yield are presented in Fig. 5. The use of 15% AA in the solvent mixture (methanol to AA = 4.25:0.75) enables the reaction to reach a FAME yield of 30.11% in 30 min, which is about 86% of that of the acid catalyzed transesterification (35%) that required a reaction of 24 h. Saka et al. (2010) proposed the kinetics of the transesterification of triglycerides in the presence of AA. At high temperature AA, a weak acid, plays the role of an acid catalyst to react with triglycerides and generate fatty acids, monoacetyl diglyceride, diacetyl mono-glyceride and triacetin. These reaction intermediates then react with methanol to produce FAME. Acid (AA) catalyzed transesterification of dry sludge was carried out at 55 °C with a solvent (methanol:AA = 4.25:0.75 mL g⁻¹) to dry sludge ratio of 30 mL g⁻¹ resulted in an FAME yield of only 2.61% in 24 h. Apparently, AA shows high catalytic activity only at high temperature. Another possible reason for the improvement on FAME yield was the enhancement of lipids extracted from sludge since lipids have better solubility in an acidic environment (Hensarling and Jacks, 1983). AA could also increase the mutual solubility of reactants in the reaction. However, reaction time longer than 30 min (Figs. 5 and 6) and more AA used (Fig. 4, methanol: AA = 4:1 mL g⁻¹) resulted in lower yields owning to possible degradation of products in acidic environment. In this study, a total time of 90 min (60 min for heating and 30 min for reaction) required is significantly shorter than studies on biodiesel production from activated sludge reported in literatures (Table 2). When pure methanol and a methanol to sludge ratio of 30 mL g⁻¹ were used, it was capable of achieving a FAME yield of 28.98% in 120 min. By using 15% AA in the AA–methanol mixture and a ratio of AA-methanol mixture to sludge of 5 mL g⁻¹, higher yield (30.11%) can be achieved in much shorter time (30 min). 3.4. Advantages of using subcritical methanol and AA Commercial biodiesel production requires the use of acid/base catalyst. Supercritical methanol method has been investigated by many researchers to avoid the use of catalysts. Nevertheless, supercritical methanol has some disadvantages such as high energy consumption and high investment of equipment since the reactions are carried out under high temperature and pressure.

Co-solvents such as CO₂ and alkane have been added in the supercritical methanol method to reduce the temperature and pressure required for the process, and to reduce the degradation of product (Encinar et al., 2012; Cao et al., 2005). Another possible way is to conduct the reaction under subcritical conditions, with minimum amount of catalysts or without catalyst, at lower temperature and
pressure than that required by the supercritical methanol method (Yin et al., 2008). In terms of reaction time and methanol amount required, the result of in situ transesterification of activated sludge in subcritical methanol and AA obtained in this study is superior to the results of studies that produced biodiesel using conventional acid catalyzed transesterification method. Dufreche et al. (2007) reported a conversion of 22.60% which is about one third of the conversion obtained in this study although the amount of methanol used is the same. However, this study can reduce the reaction time to 30 min which is much shorter than the...
24 h required in the study of Dufreche et al. (2007). Compared to the study of Huynh et al. (2012) which required a reaction time of 8 h, this study required 30 min to achieve the same conversion. Mondala et al. (2009) obtained a conversion of 97.89% which is significantly higher than that of this study (65.97%). However, a reaction time of 10 h and